Animal Feed Science and Technology 153 (2009) 101–112

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Influence of calcium oxide level and time of exposure to sugarcane on *in vitro* and *in situ* digestive kinetics

D.S. Pina[a,b], L.O. Tedeschi[a,*], S.C. Valadares Filho[b], J.A.G. Azevedo[b], E. Detmann[b], R. Anderson[c]

[a] *Texas A&M University, College Station, TX 77843, USA*
[b] *Universidade Federal de Viçosa, Viçosa, MG 36571, Brazil*
[c] *Unites States Department of Agriculture/Agricultural Research Service, Southern Plains Agricultural Research Centre, College Station, TX 77845, USA*

ARTICLE INFO

Article history:
Received 20 August 2008
Received in revised form 6 May 2009
Accepted 4 June 2009
Available online 27 June 2009

Keywords:
Calcium oxide
Gas production
Sugarcane
Digestive kinetics

ABSTRACT

These experiments were carried out to evaluate, using *in vitro* and *in situ* techniques, the effects of three inclusion levels of calcium oxide (0, 5, and 10 g/kg of sugarcane fresh matter) and four exposure times (0, 24, 48, and 72 h) of sugarcane to calcium oxide on the chemical composition and digestive kinetic parameters of sugarcane. The treatments were arranged in a 3 by 4 factorial design. Freshly-cut sugarcane (whole plant) was treated with calcium oxide and separated into 12 piles inside a barn to prevent direct exposure to sunlight, rain, and wind. Every day, before and after animal feeding, the calcium oxide was proportionally hand-mixed with approximately 150 kg of freshly-cut sugarcane to make up the dietary treatments. The lowest (Ti) and greatest (Ts) temperature and pH of the treated sugarcane piles were measured immediately before and after sampling, respectively. The ether extract (EE) and DM were not affected ($P>0.05$) by either exposure time or inclusion level. However, CP increased linearly ($P=0.01$) and OM decreased linearly ($P<0.0001$) as the exposure time and calcium oxide inclusion level increased. Interactions between inclusion level and exposure time on DM, OM, CP, EE, Ti, and Ts were not observed. However, significant interactions were detected for non-fibre carbohydrate (NFC), neutral detergent fibre (aNDF), and pH. A quadratic effect

Abbreviations: Ti, lowest temperature; Ts, greatest temperature; kd, fractional digestion rate; DM, dry matter; OM, organic matter; CP, crude protein; aNDF, neutral detergent fibre (assayed with a heat stable amylase, expressed inclusive of residual ash and without sodium sulphite); NFC, non-fibre carbohydrate; FC, fibre carbohydrate; EE, ether extract; BW, body weight.

* Corresponding author.
E-mail address: luis.tedeschi@tamu.edu (L.O. Tedeschi).

0377-8401/$ – see front matter
doi:10.1016/j.anifeedsci.2009.06.005

of exposure time on the Ti and Ts was observed (P=0.001 and P=0.001, respectively). The maximum temperature was reached with approximately 51 h of exposure time. Calcium oxide positively affected the insoluble potentially digestible fraction of sugarcane DM and aNDF (P=0.001 and P=0.001, respectively), and the indigestible fraction of sugarcane aNDF (P=0.001). Interactions between inclusion level and exposure time on soluble and indigestible fractions of sugarcane DM (P=0.0001 and P=0.01, respectively) were found. However, no interactions (P>0.27) were found between inclusion level and exposure time on aNDF digestive kinetic parameters. The fractional digestion rate (kd) of sugarcane DM and aNDF was not influenced by treatments (P>0.05). The mean values of kd for sugarcane DM and aNDF were 0.0235 and 0.0215/h, respectively. The gas production kinetics parameters were not affected (P>0.05) by treatments. In conclusion, the inclusion of calcium oxide improved the *in situ* potentially digestible fraction of sugarcane DM and aNDF; however, it did not influence the fractional digestion rate. No effects were observed on the *in vitro* digestive kinetic parameters.

1. Introduction

Sugarcane is an important alternative roughage source for ruminants under tropical feeding conditions. The high level of neutral detergent fibre (aNDF) negatively affects the intake by animals because of its slow fermentation rate, yielding a high proportion of indigestible dry matter. Therefore, alkali treatment has been proposed as an alternative to increase the digestible proportion of sugarcane aNDF, thereby providing a more available lignocellulose structure for ruminant microorganisms (Molina et al., 1983).

The artificial fibre bag (Dacron bag, nylon bag, rumen bag) technique is tool that can be used to evaluate the quality of feedstuffs and provide information about the process of degradation that occurs within the rumen. The use of the artificial fibre bag has the advantage of giving a rapid estimate of the rate and extent of the degradation of the feedstuff in a functioning rumen without the need for more complicated procedures (Ørskov et al., 1980).

The *in vitro* gas production technique has been widely used for feed evaluation and to study the kinetics of microbial fermentation processes in the rumen. This technique is based on the assumption that gas produced in batch cultures inoculated with mixed microorganisms from the rumen is directly related to the amount of substrate fermented (López et al., 2007).

The objective of these experiments was to use the *in vitro* and *in situ* techniques to evaluate the influence of the calcium oxide inclusion level and the time of sugarcane exposure to calcium oxide on the digestive kinetic parameters and the composition of sugarcane.

2. Materials and methods

2.1. Samples collection and analysis

Sugarcane samples originated from material used in a feedlot trial described by Pina et al. (2009) in which the samples were collected in 3 consecutive weeks from the material used to feed the animals. Twelve treatments from a 4 by 3 factorial design consisted of four lengths of time of sugarcane exposure to calcium oxide (0, 24, 48, and 72 h) prior to feeding and three levels of inclusion (concentration) of calcium oxide (0, 5, and 10 g of calcium oxide per kg of the sugarcane fresh matter). Every day, before feeding the animals, the freshly-cut sugarcane (whole plant approximately 150 kg from each pile) was treated with calcium oxide (finely ground), manually handling mixed, separated and stored in piles inside a barn to prevent direct exposure to sunlight, rain, and wind. These procedure was repeated every

day to obtain the dietary treatments to feeding the animals in due time. The lowest (Ti) and greatest (Ts) temperature and pH of the treated sugarcane piles were measured immediately before and after sampling, respectively. After sampling, the sugarcane samples were dried at 60 °C in a forced-air oven and ground to pass through a 2-mm screen using a Wiley mill (Model 3, Arthur H. Thomas, Philadelphia, PA). The ground samples were stored, at room temperature, in glass jars and analyzed for ether extract (EE, by loss in weight of the dry sample upon extraction with diethyl ether in a Soxhlet extraction apparatuses for 6 h; AOAC, 1990), protein (N analysis via micro Kjeldahl using 0.2 g of sample; AOAC, 1990), aNDF (Van Soest et al., 1991, assayed with a heat stable amylase, expressed inclusive of residual ash and without sodium sulphite), and ash (complete combustion in a muffle furnace at 600 °C for 6 h; AOAC, 1990). Non-fibre carbohydrates (NFC) were calculated as 100 – (%CP + %aNDF + %EE + %ash) (NRC, 2001).

2.2. In vitro procedures

The *in vitro* procedures were performed at the Ruminant Nutrition Laboratory of the Texas A&M University and at the Southern Plains Agricultural Research Centre (SPARC) of the United States Department of Agriculture in College Station, TX as described by Tedeschi et al. (2009). Briefly, rumen inocula was consistently collected from the same Holstein non-lactating cow (600 kg BW) that had access to medium quality pasture and fed dry cow ration once a day. The ruminal content was collected at the same time at the USDA/ARS (College Station, TX), and transported in a closed plastic container to the Ruminant Nutrition Laboratory (Texas A&M University, College Station, TX). Immediately upon arrival, the rumen content was filtered through four layers of cheesecloth and then through glass wool. During the filtration, the rumen fluid was continuously mixed with CO_2 to minimize changes in microbial population and to avoid O_2 contamination. At the collection, the pH and redox potential (mV) of the ruminal fluid were measured using a portable digital pH meter.

The *in vitro* medium used was the phosphate-bicarbonate buffer and reducing solution of Goering and Van Soest (1970). Samples of 200 mg of sugarcane were transferred to 125-mL Wheaton flasks that contained a Teflon-covered small stir bar. Samples were wetted with 2 mL of boiled distilled water that had been previously cooled to room temperature. The water was used to avoid particles dispersion. The medium was continuously ventilated with CO_2. The medium pH was controlled by colour change of resazurin indicator from purple to pink or colourless; the optimum pH utilized was between 6.8 and 6.9. Each flask was filled with 14 mL of medium. Strict anaerobic technique was employed in all transfers (Bryant, 1972).

The bottles were closed with previously unused butyl rubber stoppers and crimp sealed. All bottles were placed in the fermentation chamber and the respective sensor for each bottle was inserted with needles. When the fermentation chamber reached 39 °C, 4 mL of the filtered mixed ruminal bacteria inocula was injected into the bottle. The fermentation chamber was closed, and when the internal temperature reached 39 °C the pressure inside each bottle was zeroed by puncturing the stopper with a needle for approximately 5 s. The fermentation chamber was closed, and when the temperature reached 39 °C again pressure recording was initiated. The atmospheric pressure was recorded at the beginning and at the end of the fermentation.

The fermentation chamber was similar to that described by Pell and Schofield (1993). It included an incubator (chamber) with a multi-place stirrer, pressure sensors attached to the incubation flasks, an analogue to digital converter card, and a computer with software. The pressure was collected automatically by a software program every 5 min. After 48 h of fermentation 576 measurements, one every 5 min, had been taken by the computerized system.

After 48 h from the start of the fermentation process the chamber was turned off and the bottles removed. The pH and the redox potential (mV) of each bottle were measured by a portable pH meter. Subsequently, the samples were washed with 40 mL of neutral detergent solution (Van Soest et al., 1991) and cooked in an autoclave for 60 min at 105 °C. The indigestible aNDF was determined by gravimetric method, in which the insoluble neutral detergent residual was filtered through Whatman 54 filter paper, dried 48 h in an oven and weighed.

A modified dual pool logistic model with single lag value (Schofield et al., 1994) was used to fit the *in vitro* gas kinetic profiles. The first and second terms are equivalent to non-fibre (NFC) and fibre

carbohydrates pools (FC), respectively.

$$V = Vf1\left[1 + \exp\left(2 + \frac{4\mu_{m1}}{Vf1}(L - t)\right)\right]^{-1} + Vf2\left[1 + \exp\left(2 + \frac{4\mu_{m2}}{Vf2}(L - t)\right)\right]^{-1}$$

where $\mu_{m1}/Vf1$ and $\mu_{m2}/Vf2$ are specific fractional rates of digestion of each pool; $Vf1$ and $Vf2$ are maximum gas volumes achieved from completed digestion of each pool; L is the lag time; t is the time; V is the gas volume at t time; and μ_m is the maximum rate of gas production.

2.3. In situ procedures

The *in situ* experiment was carried out at the Animal Laboratory of the Animal Science Department at the Federal University of Viçosa, Brazil. Humane animal care and handling procedures followed the guidelines of the Federal University of Viçosa. Three ruminally-cannulated Nellore heifers, approximately 24 months of age with an average BW of 320 ± 20 kg, were used to evaluate the effects of levels and times of exposure calcium oxide on the *in situ* DM and aNDF digestion kinetics. The heifers were fed *ad libitum* with corn silage as the exclusive source of roughage. The concentrate (1.5 kg for each animal) was supplied once a day (8:00 in the morning) and refusals were weighed and removed every day before the next feeding. Sugarcane samples (1 g) were added to ANKOM filter bags and incubated in the rumen for 3, 6, 12, 24, 36, 48, 72, 96, and 144 h. When the bags were withdrawn from the rumen, they were soaked in water for 30 min, gently washed by hand, under running water until the washing water was clear. The zero time underwent the same procedure described above without the ruminal incubation. Bags were dried in a forced air oven at 60 °C for 72 h and weighed on a digital balance to determine the DM disappearance. Then, bags were analyzed by the ANKOM^{200} Fibre Analyzer as described by Ferreira and Mertens (2007) and the aNDF disappearance was determined by weighing bags in a digital balance after drying in an oven at 60 °C for 48 h followed by 105 °C for 8 h.

A generalized compartmental model of digestion that assumed the existence of two compartments (lag and digestion) as described by Van Milgen et al. (1991) was used to estimate the kinetic parameters of sugarcane DM and aNDF *in situ* degradation profiles. The kinetic parameters were determined by fitting the following general models:

$$\text{Residue DM} = B \times (1 + \lambda \times t) \times e^{-\lambda \times t} + C$$
$$\text{Residue aNDF} = B \times (1 + \lambda \times t) \times e^{-\lambda \times t} + C$$

where B is the insoluble potential digestible fraction, C is the indigestible fraction, λ is the rate constant that included the rate of digestion of the two compartments and t is the time of incubation. The fractional digestion rate (kd) of the digestion compartment was determined as $\lambda/2$ and the soluble fraction (A) of DM was determined by difference.

2.4. Statistical design and analysis

Statistical analyses were performed using PROC MIXED of SAS (SAS Inst. Inc., Cary, NC). The experiment was analyzed as a complete random block design in a factorial arrangement (Kuehl, 2000). The statistical model is shown below:

$$Y = \mu + \beta + c + t + c \times t + \varepsilon$$

where μ is the overall mean, β is the random effect of block, c is the level of calcium oxide inclusion, t is the time of sugarcane exposure to calcium oxide, $c \times t$ is the interaction between time of exposure and level of calcium oxide, and ε is the random error. The orthogonal contrast was used to evaluate the linear, quadratic and cubic effect of exposure time and the linear and quadratic effect of the calcium oxide inclusion level, respectively.

The nonlinear models used to determine the *in situ* and *in vitro* kinetics parameters were fitted using PROC NLIN of SAS (SAS Inst. Inc., Cary, NC).

3. Results

3.1. Sugarcane composition, pH, and temperature

The effects of the inclusion levels and exposure time to calcium oxide on the composition, pH, and temperature of sugarcane samples are shown in Table 1. Interactions between inclusion level and exposure time on DM, OM, CP, EE, Ti, and Ts were not observed. However, significant interactions were detected for NFC, aNDF, and pH (Table 1). The DM and EE were not affected ($P>0.05$) by either exposure time or inclusion level. However, CP increased linearly ($P=0.01$) and OM decreased linearly ($P<0.0001$) as exposure times and calcium oxide inclusion levels increased.

Both temperatures (Ti and Ts) were influenced by the exposure time and calcium oxide inclusion levels ($P<0.0001$ and $P<0.0001$; respectively). Quadratic effects of exposure time on the Ti ($Ti = 24.5297(\pm 1.8258) + 1.1874(\pm 0.1241) \times time - 0.0116(\pm 0.0017) \times time^2$ ($R^2 = 82.3$ and RMSE = 5.612) and Ts ($Ts = 24.1717(\pm 1.9307) + 1.2065(\pm 0.1292) \times time - 0.0119(\pm 0.0017) \times time^2$ ($R^2 = 80.7$ and RMSE = 5.942) were observed (Table 1). The maximum temperature was reached with approximately 51 h of exposure time. The effects of interaction between exposure times and calcium oxide inclusion levels on the sugarcane composition (NFC and aNDF) and pH are listed in Table 2.

3.2. In situ fermentation

Table 3 shows the effects of the inclusion level and the exposure time of sugarcane to calcium oxide on the *in situ* digestive kinetic parameters of sugarcane DM and aNDF.

The insoluble potentially digestible fractions (B) of sugarcane DM and aNDF were affected by the inclusion level of calcium oxide ($P=0.001$ and $P=0.001$, respectively) but not by the exposure time ($P=0.06$ and $P=0.14$, respectively). However, the fractional digestion rates of these fractions were neither influenced by the exposure time ($P=0.67$ and $P=0.63$, respectively) nor by the inclusion level of calcium oxide ($P=0.56$ and $P=0.62$, respectively).

The indigestible fractions (C) of sugarcane DM and aNDF were also influenced ($P=0.001$) by the inclusion level of calcium oxide although in the case of DM the effect was dependent on the exposure time as shown by the significant interaction ($P=0.01$) that was also evident ($P=0.0001$) for the soluble fraction (A) of sugarcane DM. These interaction effects are described in Table 4 together with the response contrast of all parameters significantly affected by treatments.

3.3. In vitro fermentation

Table 5 shows the effects of inclusion levels of calcium oxide and exposure time of sugarcane to calcium oxide on the *in vitro* kinetic parameters of sugarcane DM. Neither the inclusion level of calcium oxide nor the exposure time affected ($P>0.15$) the gas production kinetic parameters estimated using the dual pool logistic model (Schofield et al., 1994). Interactions between treatments were also not observed ($P>0.14$).

4. Discussion

4.1. Sugarcane composition, pH, and temperature

The spontaneous sugarcane fermentation that happens after cutting the sugarcane plant can convert up to half the soluble sugars into organic acids, reducing the quality of the sugarcane (Gonzales and Macleod, 1976). This fermentation process is accompanied by a rise in the temperature of the mass. The inclusion of calcium oxide was hypothesized to reduce the fermentation process during the storage phase of the freshly-cut sugarcane. Contrary to our hypothesis, we observed a linear increase ($P=0.001$) in the temperatures (Ti and Ts) when calcium oxide was increased. A possible explanation for this rise in the temperature is that the inclusion of calcium oxide increased the capability of the material to retain water, maintaining the heat produced during the fermentation process.

Table 1
Effect of exposure time (0, 24, 48 and 72 h) of sugarcane to calcium oxide prior to feeding and inclusion levels (0, 5 and 10 g/kg of sugarcane FM) of calcium oxide on the chemical composition, pH, and temperature of sugarcane.

Item	Exposure time (t)				P-value	Calcium oxide (CaO)			P-value	RMSE	CaO × t	Orthogonal contrast P-value (CaO/t)		
	0	24	48	72		0	5	10				Linear	Quadratic	Cubic
DM, g/kg	286	288	279	277	0.47	284	280	284	0.80	16.6	0.97	–/–	–/–	–/–
OM, g/kg of DM	962	947	942	939	0.09	965	944	922	<0.0001	7.28	0.76	<0.0001/–	0.93/–	–/–
CP, g/kg of DM	22.7	20.7	24.2	27.2	0.01	24.5	23.3	23.4	0.67	3.58	0.93	–/0.01	–/0.05	–/0.26
EE, g/kg of DM	15.3	15.1	16.8	16.1	0.06	15.4	16.0	16.0	0.47	1.31	0.51	–/–	–/–	–/–
NFC, g/kg of DM	444	439	401	380	<0.0001	433	420	394	0.01	27.1	0.001	–/–	–/–	–/–
aNDF, g/kg of DM	464	473	500	515	0.0002	492	485	488	0.72	22.0	0.0001	–/–	–/–	–/–
pH	9.63	6.04	4.74	4.81	<0.0001	4.18	6.64	8.09	<0.0001	0.32	<0.0001	–/–	–/–	–/–
Ti, °C	23.9	48.3	52.1	50.4	<0.0001	39.3	44.6	47.1	0.0001	3.60	0.46	0.001/0.001	0.31/0.001	–/0.01
Ts, °C	24.1	46.6	54.4	49.6	<0.0001	38.1	45.6	47.3	<0.0001	3.31	0.34	0.001/0.001	0.20/0.001	–/0.67

DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; NFC = non-fibre carbohydrate; aNDF = neutral detergent fibre; Ti = lowest temperature; Ts = greatest temperature; RMSE = root mean square error.

Table 2
Evaluation of interaction effects between exposure time (0, 24, 48 and 72 h) of sugarcane to calcium oxide prior to feeding and calcium oxide inclusion levels (0, 5 and 10 g/kg of sugarcane FM) on NFC (g/kg of DM), aNDF (g/kg of DM), and pH of sugarcane.

Item	Exposure time				Calcium oxide			Contrast (*P*-value)		
	0	24	48	72	0	5	10	Linear	Quadratic	Cubic
NFC										
0 g/kg	401	463	457	380	–	–	–	0.75	0.002	0.68
5 g/kg	462	442	382	393	–	–	–	0.001	0.32	0.12
10 g/kg	468	410	364	335	–	–	–	<0.0001	0.36	0.93
0 h	–	–	–	–	401	462	468	0.01	0.17	–
24 h	–	–	–	–	463	442	410	0.03	0.79	–
48 h	–	–	–	–	457	382	364	0.001	0.15	–
72 h	–	–	–	–	380	393	335	0.002	0.31	–
aNDF										
0 g/kg	521	470	469	507	–	–	–	0.44	0.002	0.86
5 g/kg	448.	472	516	502	–	–	–	0.002	0.16	0.18
10 g/kg	421	477	517	537	–	–	–	<0.0001	0.18	0.97
0 h	–	–	–	–	521	448	421	<0.0001	0.15	–
24 h	–	–	–	–	470	472	477	0.69	0.89	–
48 h	–	–	–	–	469	516	517	0.01	0.15	–
72 h	–	–	–	–	507	502	537	0.10	0.22	–
pH										
0 g/kg	5.75	3.73	3.63	3.62	–	–	–	<0.0001	<0.0001	0.04
5 g/kg	11.3	5.76	4.80	4.75	–	–	–	<0.0001	<0.0001	0.002
10 g/kg	11.9	8.63	5.80	6.06	–	–	–	<0.0001	<0.0001	0.004
0 h	–	–	–	–	5.75	11.3	11.9	<0.0001	<0.0001	–
24 h	–	–	–	–	3.73	5.76	8.63	<0.0001	0.08	–
48 h	–	–	–	–	3.63	4.80	5.80	<0.0001	0.71	–
72 h	–	–	–	–	3.62	4.75	6.06	<0.0001	0.71	–

NFC = non-fibre carbohydrate; aNDF = neutral detergent fibre.

This explanation is agreement with the report by Freitas et al. (2007) who found a linear decrease of the sugarcane DM with increasing inclusion levels of calcium oxide (2.5, 5, 10, 20, and 40 g/kg of sugarcane fresh matter). However, in this experiment we found no (P=0.80) effects of the calcium oxide inclusion level on the sugarcane DM likely due to a lower level of calcium oxide inclusion used in this trial as compared those used by Freitas et al. (2007). Therefore, the increase on the temperature could be due to a more intense fermentation process observed as calcium oxide inclusion increased, which was indicated by the reduction of NFC content of sugarcane (Table 2).

Interactions between inclusion level and exposure time on NFC, aNDF, and pH (P=0.001, P=0.0001, and P<0.0001, respectively) were observed. The sugarcane aNDF content was decreased linearly (P<0.0001) and increased linearly (P=0.01) by the calcium oxide inclusion level at the 0 and 48 h exposure times, respectively. However, no effects (P>0.05) were found for calcium oxide inclusion level at the 24 and 72 h exposure times.

Decrease in the aNDF content while increasing the alkali inclusion has been reported for other roughage sources, mainly cereal straws (Molina et al., 1983). These results were likely due to a solubilisation of hemicellulose because the ADF fraction is not significantly affected by alkali treatment (Wilkinson and Gonzalez Santillana, 1978). The decrease in aNDF content of rhodes grass hay as calcium oxide inclusion increased was reported by Granzin and Dryden (2003). Freitas et al. (2007) evaluated the effect of calcium oxide inclusion (2.5, 5, 10, 20, and 40 g/kg of sugarcane fresh matter) on the sugarcane nutritional quality, and observed a decrease in the aNDF content as the calcium oxide inclusion was increased, with 0 or 9 h of exposure time. These results are in agreement with those found in our trial.

On the other hand, the increase in the sugarcane aNDF content that resulted from the inclusion of calcium oxide for the 48 h exposure time could be due to a counter effect of increased exposure time on the of sugarcane aNDF content, which could be due to leaching or fermentation of soluble carbohydrates as the exposure time increased.

Table 3
In situ ruminal digestive kinetic parameters of sugarcane treated with different levels of calcium oxide (0, 5 and 10 g/kg of sugarcane fresh basis) and different times of exposure (0, 24, 48 and 72 h) to calcium oxide prior to feeding.

Item	Exposure time (t)				P-value	Calcium oxide (CaO)			P-value	RMSE	CaO × t
	0	24	48	72		0	5	10			
DM											
A, g/kg of DM	557	553	527	525	0.001	540	545	536	0.51	18.7	0.0001
B, g/kg of DM	188	155	173	178	0.06	157	167	198	0.001	24.3	0.27
kd, /h	0.0254	0.0240	0.0233	0.0212	0.67	0.0249	0.0238	0.0217	0.56	0.01	0.83
C, g/kg of DM	255	292	300	297	0.001	303	288	266	0.001	22.4	0.01
aNDF											
B, g/kg of aNDF	449	401	407	418	0.14	384	405	466	0.001	44.9	0.27
kd, /h	0.0230	0.0204	0.0223	0.0205	0.63	0.0225	0.0216	0.0205	0.62	0.01	0.80
C, g/kg of aNDF	551	599	593	582	0.14	616	595	534	0.001	44.9	0.27

A = soluble fraction; *B* = insoluble potentially digestible fraction; kd = fractional rate of digestion and *C* = indigestible fraction. RMSE = root mean square error.

Table 4
Evaluation of linear and quadratic components of significant main effects and interaction between exposure time (0, 24, 48 and 72 h) of sugarcane to calcium oxide prior to feeding and calcium oxide inclusion levels (0, 5 and 10 g/kg of sugarcane fresh basis) on the *in situ* aNDF and DM (*A* and *C*, g/kg of DM) fermentation kinetic parameters of sugarcane.

Item	Exposure time (*t*)				Calcium oxide (CaO)			Contrast (*P*-value)		
	0	24	48	72	0	5	10	L	Q	C
DM										
B, g/kg of DM	–	–	–	–	157	167	198	0.001	0.26	–
A										
0 h	–	–	–	–	517	556	598	0.001	0.90	–
24 h	–	–	–	–	573	564	523	0.01	0.25	–
48 h	–	–	–	–	542	521	519	0.15	0.49	–
72 h	–	–	–	–	529	540	505	0.12	0.10	–
0 g/kg	517	573	542	529	–	–	–	0.91	0.01	0.04
5 g/kg	556	564	521	540	–	–	–	0.07	0.63	0.04
10 g/kg	598	523	519	505	–	–	–	0.001	0.01	0.10
C										
0 h	–	–	–	–	306	258	200	0.001	0.78	–
24 h	–	–	–	–	311	290	274	0.04	0.89	–
48 h	–	–	–	–	290	313	297	0.67	0.24	–
72 h	–	–	–	–	306	291	294	0.51	0.57	–
0 g/kg	306	311	290	306	–	–	–	0.72	0.65	0.28
5 g/kg	258	290	313	291	–	–	–	0.06	0.05	0.57
10 g/kg	200	274	297	294	–	–	–	0.0001	0.01	0.70
aNDF										
B, g/kg of aNDF	–	–	–	–	384	405	466	0.001	0.22	–
C, g/kg of aNDF	–	–	–	–	616	595	534	0.001	0.22	–

A = soluble fraction; *B* = insoluble potentially digestible fraction; kd = fractional rate of digestion and *C* = indigestible fraction.

The sugarcane NFC content had a quadratic response (P=0.002) with the exposure time on the null calcium oxide inclusion level. However, a linear decrease (P=0.001 and P<0.0001) on the sugarcane NFC content as exposure time increased was observed when calcium oxide was included at 5 or 10 g/kg. Conversely, an inverse relationship was observed with aNDF. The possible reason for this result is that the NFC was determined through a summative equation that included the aNDF as a component. So any variation in the aNDF content would impact the NFC content. Other reason could be the fermentation process that consumes the soluble carbohydrate contents of the material that is included on the NFC fraction and convert it into gas and consequently generating heat. As observed in Table 1, the temperature of sugarcane samples was increased by both exposure time and calcium oxide inclusion level. This could be an indication that fermentation takes place during storage of material.

The sugarcane NFC content was increased linearly (P=0.01) by the calcium oxide inclusion level for the 0 h exposure time. However, the sugarcane NFC content was linearly decreased for the exposure times of 24, 48, and 72 h (P=0.03, P=0.001, and P=0.002, respectively). The increase in the sugarcane NFC content due to the calcium oxide inclusion level for the 0 h exposure time was likely due to the decrease in the aNDF content. However, the decrease in the NFC content for the exposure times of 24, 48, and 72 h as calcium oxide inclusion level increased could be due to a linear increasing and decreasing effect of exposure time on the aNDF and NFC content within the calcium oxide inclusion levels of 5 and 10 g/kg of sugarcane fresh matter, respectively (Table 2).

The sugarcane pH was linearly decreased (P<0.0001) as exposure time increased regardless of the calcium oxide inclusion level. However, as expected a linear increase (P<0.0001) in the sugarcane pH was observed for each calcium oxide inclusion level regardless of the exposure time. The increase in pH with alkali treatments and decrease with exposure time are in agreement with the results obtained by Molina et al. (1983). The reduction of pH as exposure time increased could be due to a partial neutralization of alkali by organic acids formed during the fermentation process. As discussed by Gonzales and Macleod (1976) the spontaneous sugarcane fermentation that happened after the plants

were cut and ground could result in up to half of the soluble sugars being converted into organic acids.

4.2. In situ fermentation

The mean values of fractional digestion rates of sugarcane DM insoluble but potentially digestible and aNDF were 0.0235 and 0.0215/h; respectively (Table 3). As observed in Table 1, approximately 480 and 460 g/kg of sugarcane OM were composed of aNDF and NFC, respectively. The sugarcane NFC is almost 100% of soluble sugars (sucrose). This fact is in agreement with the mean sugarcane DM soluble fraction of 541 g/kg. Consequently, the remaining sugarcane DM insoluble potentially digestible fraction is composed basically by insoluble potentially digestible aNDF, which could explain the similar rates of fermentation observed in this experiment for DM and aNDF (0.0235 and 0.0215/h).

As observed in Table 4, the calcium oxide inclusion level increased linearly (P=0.001) the insoluble potentially digestible aNDF, and consequently decreased linearly (P=0.001) the aNDF indigestible fraction. Similar to aNDF, the calcium oxide inclusion level resulted in a linear increase (P=0.001) in the insoluble potentially digestible sugarcane DM fraction as can be expected from the large contribution of aNDF to this fraction. Therefore, the effect of calcium oxide inclusion level on the insoluble potentially digestible sugarcane aNDF was reflected in the insoluble potentially digestible sugarcane DM fraction. The aNDF fractional digestion rate was found by Henriques et al. (2007) to increase with calcium oxide inclusion level (0, 5, 10, 15, and 20 g/kg of sugarcane fresh matter), ranging from 0.0267 to 0.0295/h. These values are greater than the ones found in our trial, which ranged from 0.0205 to 0.0230/h without significant differences between treatments. Henriques et al. (2007) also reported a positive effect of calcium oxide on the potentially digestible aNDF fractions, with values ranging from 498 to 605 g/kg.

The increase in the sugarcane potentially digestible aNDF fraction could be due to an effect of the alkali treatment on cell wall constituents. Cellulose swells have been reported by Whistler and Teng (1970) as the result of alkali reducing the strength of the inter-molecular hydrogen bonds that bind cellulose molecules. In addition, the inter-molecular ester linkages between uronic acid groups of hemicelluloses are probably hydrolyzed as well (Feist et al., 1970). This observation is in agreement with the study by Morris and Bacon (1976), who observed that the increase in hemicellulose digestibility was probably due to the release of phenyl and acetyl groups, especially abundant in graminae. As suggested by Jackson (1977), the swollen cellulose should be more easily attacked by ruminal bacteria, increasing the aNDF or DM potentially digestible fractions.

As reported by Allen and Mertens (1988), fibre represents a significant fraction of the diets of ruminants; thus, an animal's performance is related to its ability to consume and digest the fibrous portion of its diet. The fibre fraction that is resistant to digestion and the rate of digestion and passage of potentially fermentable fibre are important factors that constrain digestion of fibre in the rumen, and can interfere with the DMI through the rumen fill effect. Therefore, treatments that reduce the indigestible fraction of fibre should positively impact animal DMI and performance.

The effects of calcium oxide inclusion level and exposure time of sugarcane to calcium oxide on the soluble (A) and indigestible (C) DM fractions of sugarcane are described in Table 4. The inclusion level of calcium oxide increased the soluble fraction linearly (P=0.001) at 0 h of exposure time but the gain in soluble was lost after 24 or 48 h of exposure time for the 10 and 5 g/kg of alkali treatment respectively, probably due to the utilization of this readily fermentable fraction. This effect plus the solubilisation of DM in the non-treated sugarcane (as shown by the quadratic pattern of time on fraction A and NFC content; Table 2) might have reversed the response after 24 h of exposure time. As expected, an inverse relationship was observed for the indigestible fraction, which decreased linearly (P=0.001) in response to calcium oxide treatment at 0 h of exposure time but increased linearly (P=0.04) after 24 h of exposure time.

4.3. In vitro fermentation

The use of the dual pool logistic model (Schofield et al., 1994) to fit the sugarcane gas production profiles rather than a one pool model is justifiable because sugarcane has at least two different well-

Table 5
In vitro kinetic parameters of sugarcane treated with different levels of calcium oxide (0, 5, and 10 g/kg of sugarcane fresh basis) and different times of exposure (0, 24, 48 and 72 h) to calcium oxide prior to feeding.

Item	Exposure time (t)				P-value	Calcium oxide (CaO)			P-value	RMSE	CaO × t
	0	24	48	72		0	5	10			
$Vf1$, mL/100 g of DM	10.2	10.6	10.1	10.5	0.93	10.5	10.3	10.3	0.95	1.82	0.69
$\mu_{m1}/Vf1$, mL gas/h	0.263	0.212	0.243	0.190	0.60	0.238	0.195	0.247	0.54	0.12	0.14
L, h	1.65	1.76	1.97	1.45	0.18	1.87	1.53	1.72	0.27	0.49	0.70
$Vf2$, mL/100 g of DM	9.6	8.9	7.7	8.7	0.33	9.2	8.2	8.8	0.55	2.19	0.95
$\mu_{m2}/Vf2$, mL gas/h	0.0496	0.0596	0.0429	0.0557	0.77	0.0688	0.0412	0.0458	0.15	0.036	0.95

$\mu_{m1}/Vf1$ and $\mu_{m2}/Vf2$ = specific fractional rates of digestion of NFC and FC, respectively. $Vf1$ and $Vf2$ = maximum gas volumes achieved from digestion of NFC and FC, respectively. L = lag time. RMSE = root mean square error.

defined sources of carbohydrates (NFC and FC) that have very different fractional digestion rates, as can be observed in Table 5. According to Schofield (2000), the multiple pool approach is appealing because the chemical assay have been devised to measure separated carbohydrates pools (like aNDF, ADF, and NFC), which are digested at different rates *in vitro* and they are also conceptually simpler to understand.

Fernandes et al. (2003) evaluated sugarcane digestive kinetic parameters by fitting the dual pool logistic model (Schofield et al., 1994) to gas production profiles and reported mean fractional digestion rates of 0.184 and 0.0227/h for NFC and FC, respectively. These values are smaller than the ones found in our study (0.227 and 0.0519/h, respectively). However, Fernandes et al. (2003) found a mean total gas volume production of 26.8 mL/100 mg of DM, which was higher than the 19.1 mL/100 mg of DM found in our study. Digestive kinetic parameters of three different sugarcane varieties were evaluated by Azevêdo et al. (2003) by fitting the dual pool logistic model (Schofield et al., 1994). They reported mean fractional digestion rates of 0.291 and 0.0267/h for NFC and FC, respectively, and also reported a mean total gas volume production of 17.2 mL/100 mg of DM and a mean lag time of 3.43 h, which were lower and higher than 19.1 mL/100 mg of DM and 1.71 h, respectively.

The differences among the experiments could be due to variability between the sugarcane varieties use by Fernandes et al. (2003), Azevêdo et al. (2003), and that one used in our trial, and the absence of a clear relationship between fractional digestion rate and total gas production volume was reported by Beuvink and Spoelstra (1992), who found no clear relationship between these parameters. Therefore, the gas production digestive kinetics parameters found in this trial suggested the absence of an effect of levels of inclusion of calcium oxide on the *in vitro* microbial activity. The smaller value to the lag time (1.71 h) reported in this trial than that one (3.43 h) reported by Azevêdo et al. (2003) could be due to a fast colonization and fermentation of the samples. The mean total gas volume (19.1 mL/100 mg) obtained could indicate a normal microbial development under gas production technique.

5. Conclusion

The inclusion of calcium oxide improved the *in situ* potentially digestible fraction of sugarcane DM and aNDF. However, it did not influence the fractional digestion rate, and no effects were observed on the *in vitro* digestive kinetic parameters. The calcium oxide inclusion level and the exposure time negatively affected the sugarcane NFC content and the level of calcium oxide used in this experiment could not prevent that the fermentation process took place on the *in nature* sugarcane.

Acknowledgements

We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) and the Fundação de Apoio à Pesquisa de Minas Gerais (FAPEMIG, Brazil) for financial support. Appreciation is extended to the staff of the Animal Science Department of the Federal University of Viçosa for their assistance in conducting this experiment, and to students and staff of the Ruminant Nutrition Laboratory at Texas A&M University.

References

AOAC, 1990. Official Methods of Analysis, 15th ed. Assoc. Offic. Anal. Chem., Arlington, VA.
Allen, M.S., Mertens, D.R., 1988. Evaluating constraints on fiber digestion by rumen microbes. J. Nutr. 118, 261–270.
Azevêdo, J.A.G., Pereira, J.C., Queiroz, A.C., Carneiro, P.C.S., Lanna, R.P., Barbosa, M.H.P., Fernades, A.M., Rennó, F.P., 2003. Chemical–bromatological composition, fractionation of carbohydrates and in vitro fiber degradation kinetics of three sugarcane (*Saccharum* spp.) varieties. Rev. Bras. Zootecnia 32, 1443–1453.
Beuvink, J.M.W., Spoelstra, S.F., 1992. Interactions between substrate, fermentations end-products, buffering systems and gas production upon fermentation of different carbohydrates by mixed rumen microorganisms in vitro. Appl. Microbiol. Biotechnol. 37, 505–509.
Bryant, M.P., 1972. Commentary on the Hungate technique for culture of anaerobic bacteria. Am. J. Clin. Nutr. 25, 1324–1328.
Feist, W.C., Baker, A.J., Tarkow, H., 1970. Alkali requirements for improving the digestibility of hardwoods by rumen microorganisms. J. Anim. Sci. 30, 832–835.
Ferreira, G., Mertens, D.R., 2007. Measuring detergent fiber and insoluble protein in corn silage using crucibles or filter bags. Anim. Feed Sci. Technol. 133, 335–340 (Short communication).
Fernandes, A.M., Queiroz, A.C., Pereira, J.C., Lana, R.P., Barbosa, M.H.P., Fonseca, D.M., Detmann, E., Cabral, L.S., Pereira, E.S., Vittori, A., 2003. Fractionation and in vitro degradation kinetics of carbohydrates constituents of sugarcane with different cycles of production and three cut time. Rev. Bras. Zootecnia 32 (Suppl. 1), 1778–1785.
Freitas, A.W.P., Rocha, F.C., Fagundes, J.L., Fonseca, R., 2007. Nutritional quality of sugar cane treated with calcium oxide. J. Anim. Sci. 85 (Suppl. 1), 347.
Goering, H.K., Van Soest, P.J., 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). In: Agric. Handbook No. 379. ARS-USDA, Washington, DC, pp. 1–20.
Gonzales, E., Macleod, N.A., 1976. Spontaneous fermentation of sugarcane. Trop. Anim. Product. 1, 80–85.
Granzin, B.C., Dryden, G.McL., 2003. Effects of alkalis, oxidants and urea on the nutritive value of Rhodes grass (*Chloris gayana* cv. Callide). Anim. Feed Sci. Technol. 103, 113–122.
Henriques, L.T., Valadares Filho, S.C., Detmann, E., Pina, D.S., Costa, V.A.C., Fonseca, M.A., Benedeti, P.D.B., Diniz, L.L., 2007. Chemical composition and neutral detergent fibre in situ degradation of sugarcane treated with calcium oxide under different inclusion levels and time of exposure. In: Proceedings of the XVII Brazilian Meeting of Animal Science, IV International Meeting of Animal Science, Londrina-PR (CD-ROM).
Jackson, M.G., 1977. Reviews article the alkali treatment of straws. Anim. Feed Sci. Technol. 2, 105–130.
Kuehl, R.O., 2000. Design of Experiments: Statistical Principles of Research Design and Analysis, 2nd ed. Duxbury Press, New York.
López, S., Dhanoa, M.S., Dijkstra, J., Bannink, A., Kebreab, E., France, J., 2007. Some methodological and analytical considerations regarding application of the gas production technique. Anim. Feed Sci. Technol. 135, 139–156.
Molina, E., Boza, J., Aguilera, J.F., 1983. Nutritive value for ruminants of sugar cane bagasse ensiled after spray treatment with different levels of NaOH. Anim. Feed Sci. Technol. 9, 1–17.
Morris, E.J., Bacon, J.S.D., 1976. Digestion of acetyl groups and cell wall polysaccharides of grasses in the tureen. Proc. Nutr. Soc. 35, 94A.
NATIONAL COUNCIL RESEARCH – NRC, 2001. Nutrient Requirements of Dairy Cattle, 7th ed. National Academic Press, Washington, DC, p. 381.
Ørskov, E.R., DeB Hovell, F.D., Mould, F., 1980. The use of nylon fiber bag technique for the evaluation of feedstuffs. Trop. Anim. Prod. 5 (3), 195–213.
Pell, A.N., Schofield, P., 1993. Computerized monitoring of gas production to measure forage digestion in vitro. J. Dairy Sci. 76, 1063–1073.
Pina, D.S., Valadares Filho, S.C., Tedeschi, L.O., Detmann, E., Azevêdo, J.A.G., Barbosa, A.M., 2009. Effects of CaO level and time of exposure to sugarcane on the digestive parameters and performance of Nellore heifers. Rev. Bras. Zootecnia, in press.
Schofield, P., 2000. Gas production methods. In: D'Mello, J.P.F. (Ed.), Farm Animal Metabolism and Nutrition. CABI Publishing, pp. 209–232 (Chapter 10).
Schofield, P., Pitt, R.E., Pell, A.N., 1994. Kinetics of fiber digestion from in vitro gas production. J. Anim. Sci. 72, 2980–2991.
Tedeschi, L.O., Kononoff, P.J., Karges, K., Gibson, M.L., 2009. Effects of chemical composition variation on the dynamics of ruminal fermentation and biological value of corn milling (co)products. J. Dairy. Sci. 92 (1), 401–413.
Van Milgen, J., Murphy, M.R., Berger, L.L., 1991. A compartmental model to analyse ruminal digestion. J. Dairy Sci. 74, 2515–2529.
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3590.
Whistler, R.L., Teng, J., 1970. Cellulose chemistry. In: Britt, K.W. (Ed.), Handbook of Pulp and Paper Technology, 2nd ed. Van Nostrand Reinhold, New York, pp. 13–23.
Wilkinson, J.M., Gonzalez Santillana, R., 1978. Ensiled alkali-treated straw. I. Effect of level and type of alkali on the composition and digestibility in vitro of ensiled barley straw. Anim. Feed Sci. Technol. 3, 117–132.